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EDITORIAL

ANATOMICAL RESEARCH AND EVOLUTION

THERE is evidence in historical record that British horses were small in size. The native breeds of the present day are supposed to be descended from this original stock. We know also that a few hundred years ago, a relatively short period in the march of time, steps were taken to develop and increase the size and power of our horses. The great draught horses, the racehorses and the hunter types of which we are so proud are the result of a sagacious importation policy coupled with a selective cross-mating. In spite of this certain native breeds of ponies exist to this day. They differ considerably in form, size and even character and they serve as a living example of nature's law or rule, i.e., "Adaptation to environment."

There is no doubt that by accident or design some intermixing of blood must have occurred in the past, but there can be no doubt that these so-called native breeds are the nearest of all our horses to the original type. Many authorities consider that the Exmoor breed have the purest blood of all, and this has led to a very healthy controversy for and against the idea.

It is not surprising to learn that no scientific record exists dealing with the anatomy and origin of the Exmoor or, indeed, of any other native breed. During the past three years the Exmoor pony has been the subject of research carried out by J. G. Speed and M. G. Etherington in the Anatomy Department of the Edinburgh Veterinary School. In this issue, and under the title, "An Aspect of the Evolution of British Horses," they record some of their findings and conclusions. The work has entailed a study of the archæological and palæontological evidence of the continuous existence of horses in this country,

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and it demonstrates also the wide application of the science of Anatomy. This knowledge has been used to identify the relationship between some of our modern horses and their prehistoric ancestors. If the prototypes from which our modern specialised animals are said to be descended can be identified with certainty, then it may be possible to demonstrate the anatomical and genetical potentialities contained within present-day stock. Such knowledge may enable us to recognise when and where errors in selective breeding have occurred and we may be able to remedy or compensate for the past and avoid trouble in the future.

It may be assumed that the indigenous breeds which have evolved naturally in any country have, by the hard test of " the survival of the fittest," adapted themselves to the economy of their environment, and that when man has taken a hand in the process he has attempted to increase their productivity and usefulness without too great a loss of economy. When one considers the state of our modern animals one has doubts if man's influence has been completely beneficial and whether productivity and economy have maintained a proper balance.

A new line of research is welcome and refreshing. Speed and Etherington have aimed at establishing the origin of a specific breed of livestock and demonstrating those anatomical factors which have made for survival against the adverse competition of nature and man. They postulate the sources and causes which have been responsible for aberrations and defects which influence the health, longevity and economy of the modern product.

This work may have repercussions far beyond the Exmoor breed of pony. If their evidence and theories are substantiated then a new era in livestock production may open up and the breeding plans of the future be based upon proved scientific standards rather than upon the empirical methods of trial and error of the past.

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GENERAL ARTICLES

AN ASPECT OF THE EVOLUTION OF BRITISH HORSES

By J. G. SPEED and M. G. ETHERINGTON,

Department of Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh.

THE origin of the horse has received considerable attention in North America, where fairly complete series of fossils, beginning with Eohippus, have been preserved by the fortuitous chances of geography. The American Museum of Natural History has handled this material so competently, and has exhibited it and classified it so carefully, that an impression, perhaps erroneous, has been gained popularly that all horses originated in North America.

In Europe, particularly in Germany and the Netherlands, this evolution of the early horses in North America is accepted, but an attempt is made to claim for North Africa, Asia, and Germany, the honour of being the homes of Modern Horses. Although this European claim is probably in part true, it falls far short of explaining some of the most important problems. The general understanding is that horses evolved in North America from Eohippus, which had four toes on its fore-feet and three toes on its hind, and which lived in the subtropical conditions of the age of early mammals, perhaps as early as 70 million years ago. It was a small animal, probably weighing about 10 to 15 lb.

The stages of horse evolution over the subsequent 50 million years tended to produce the three-toed horses, varying in size from that of a sheep to about 10 hands. Finally, about 15 million years ago, the one-toed horses began to evolve, although some three-toed horses survived and were probably more numerous than the one-toed, and one of them, Hipparion, lived until, perhaps, 500,000 years ago, or even later. We may accept a North American origin purely on the evidence that numerous fossils exist there, but if we do, we must explain why the oldest and most primitive horse-fossils known, those of Hyracotherium, have been found in the South of England. Another point of interest is that one of the three-toed horses, Ancitherium, has been found in Europe, but not in America.

The British Isles and North America were part of the same continent in the Jurassic period and maintained land connections over what is now the North Atlantic for a very long time after, in fact, throughout much of the Tertiary period. Britain was then a large land area, but underwent so much erosion that most of the geological deposits of this Tertiary era have since been lost. By the time that the land connections with North America had been covered by the ocean, or even before, Britain had become joined to France. This geographical history seems to be more or less overlooked in popular understanding of

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the evolution of the horse, but it is really necessary to realise that Britain and its surrounding land mass played a most important part in the development of the horse, and may very well have been the source from which North America was colonised by early horses. Presumptive evidence may go even further and may indicate that the evolution of some horses may have been completed in or near Britain.

This very superficial sketch of its evolution in the Tertiary era may serve as an introduction to an abstract study of the horse, but it is when we come to the Quaternary era that the interests of the veterinary surgeon and the stock-breeder become involved.

The Quaternary era covers approximately the last million years, of which the first part is called the Pleistocene period, during which the ice ages occurred, and the last 10,000 years is the Holocene period.

No great success has attended efforts to identify the races or subspecies of *Equus caballus* which could have been the founders of modern horse breeds. Just as in the Tertiary horses there were many species and subspecies of the different genera, so, in the Pleistocene horses there were several species, such as the zebras, asses, and quaggas, as well as the true horses, and the true horses certainly included numerous subspecies.

There are two opinions commonly expressed : one is that all the Pleistocene horses migrated from North America, with their development already completed, across the region of the Behring Straits into Asia; the other is that the Tertiary horses came from America to Asia by this route, and in Europe, Asia and Africa developed into " cold-blooded " horses, Tarpans and Prjevalsky wild horses, and Arab horses. On this last theme there are several variations, and sometimes the cold-blooded horses are subdivided into *Equus germanicus* and Microhippus (the latter being the ponies).

If the classification of the Pleistocene horses is varying and confused, that of the Holocene or post-glacial is even more difficult of comprehension. American horses began to die out, although they seemed to have survived in the Pampas until about three thousand years ago. In Europe, at least two races of ponies still alive to-day have survived throughout the Holocene, and for some considerable period back into the Pleistocene, practically unchanged. Britain and France may be considered together as the home of these ponies, and fortunately for our researches, plenty of Pleistocene fossils and Palæolithic cave drawings are available in these countries, with which to compare the living prototypes. In saying this we are aware that we are contradicting the generally expressed view that European wild horses became extinct during the early phase of the age of Man.

The Asiatic wild horse, *Equus C. Prjevalsky* may still exist in the Gobi and we are fortunate in having its skeleton for our comparisons. The Tarpan appears, from such evidence as we have obtained, to be a cross-bred horse, probably derived from Mongolian Arabs, but so far we have only seen photographic evidence of this. The European ponies, and Prjevalsky's horse, appear to have

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PLATE 1

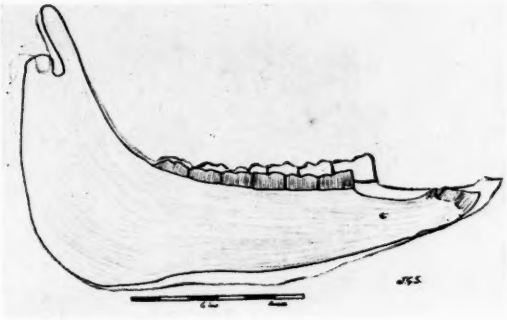

FIG. 1
Outline of Prjevalsky mandible, and an Exmoor pony jaw (shaded).

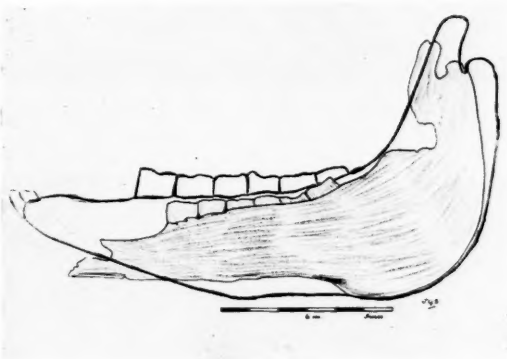

FIG. 2
Outline of Prjevalsky mandible with, Circ. 250 B.C. Arab-type mandible from a Celtic Burial near Reading (shaded).

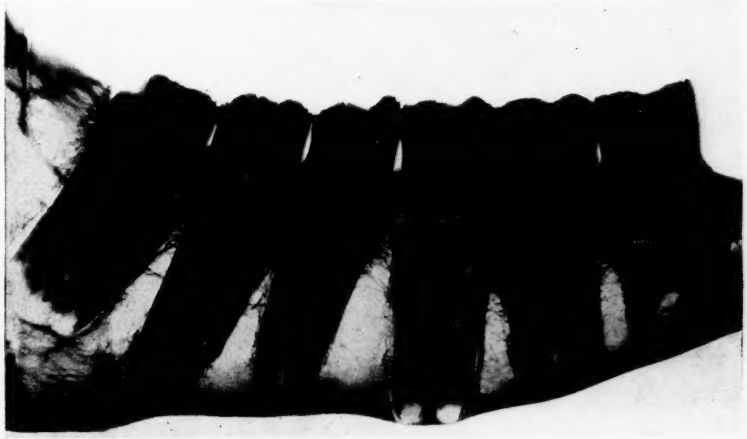

FIG. 3
X-ray of mandible of a 5-year-old Prjevalsky mare.

(Article by Speed and Etherington, page 147)

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FIG. 4
Mandible of a 4½-year-old Exmoor pony mare.

FIG. 5
Mandible of the Howenegg Hipparion gracile.

(*Article by Speed and Etherington, page* 147)

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(*Article by Speed and Etherington, page 147*)

FIG. 6
An aged Arab-type stallion Circ. 250 B.C.

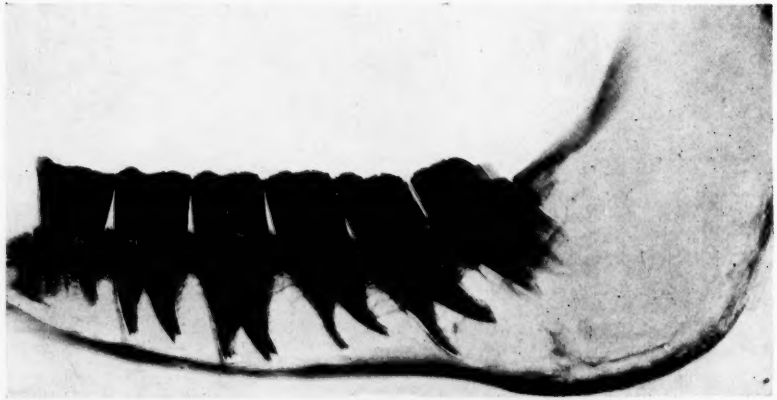

FIG. 7
A Javanese pony—6 years old—A.D. 1900.

FIG. 8
A cross-bred pony showing the results of crowded implantation of the teeth.

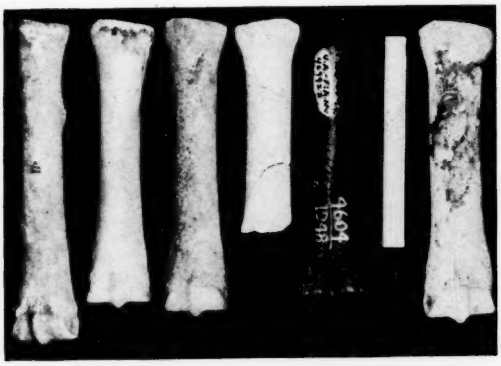

FIG. 9
Metacarpels. Left to right: (a) From the Roman Signal Station site, Scarborough; (b) Exmoor pony mare, A.D. 1947; (c) Found near a Brigantes Settlement, Piercebridge; (d) From the Mantell Collection of Chalk conglomerate fossils, Circ. B.C. 100,000; (e) From Alaskan Pleistocene river gravels; (f) The larger type of Pleistocene pony, from Wookey Hole, Circ. B.C. 60,000.

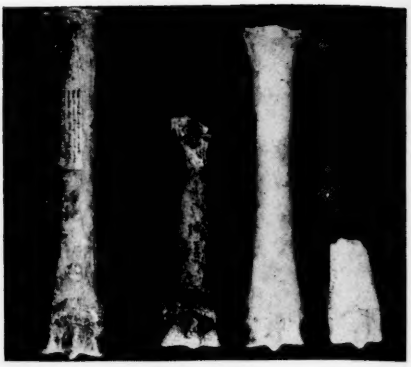

FIG. 10
Metatarsals. Left to right: (a) The larger type of Pleistocene European pony—from the Mendip Caves; (b) The smaller type of Pleistocene European pony—from the Mendip Caves; (c) An Exmoor pony mare, A.D. 1947; (d) The smaller type of Pleistocene European pony—from the Dordogne, France—Circ. 30,000 B.C.

FIG. 11
Northern horses are not extinct. A herd of prototype Exmoor ponies on the Pentland Hills near Edinburgh. November, 1951.

FIG. 12
A Northern stallion.

(*Article by Speed and Etherington, page* 147)

been derived from the true Northern Pleistocene horses, and when not crossed with Arabs, have certain distinct dental characters. The modern Arab horses are an extremely variable group as far as their dental characters are concerned, and give evidence of much crossing with the Northern animals, but the oldest prototype specimen we have managed to obtain, a three thousand-year-old mandible, has given us a fair indication of the original dental characters of the Arabs.

To understand the significance of these dental variations it is necessary to survey the conditions that have obtained during the Pleistocene and Holocene periods.

Horses of the Pleistocene era could live in the tundra and steppes as grazing animals, or they could live in a subtropical environment nearer the equator. The Pleistocene was the period of dominance of horses, to judge by the great numbers of bones and fossils. During the Pleistocene there have been advances and regressions of the arctic polar ice cap with consequent increase or decrease of the areas of grasslands. Throughout the whole period, latitude 45°-50° appears to have been the optimum region for horse survival, in Asia, Europe, and North America. The horses typical of these Northern grasslands show remarkable similarity in tooth development, long-crowned, straight in their length, and with a high proportion of reserve crown. The enamel pattern of the grinding surface of the cheek teeth is complex, and in young adults, wavy or curved enamel ridges surround the infundibuli. A characteristic of their implantation in the jaws is that the reserve crowns and roots diverge, or conversely that the erupted crowns converge so that the axes of these teeth could be projected to meet together at one point. This may be taken as typical of the true grazing horses. Their mandibles are deep and their maxillæ high.

Such horses have incisor teeth which are strongly curved so that the upper and lower rows meet at an obtuse angle, and consequently grass can be bitten off cleanly. Even in old age, and Northern horses may be expected to live from 30-40 years, this cupping of the incisor teeth remains, because the bones which carry the incisors, the body of the mandible in particular, are also curved, not horizontal as in the Arabs.

Northern horses are not extinct. On the contrary it may be demonstrated that some of the modern ponies at least, are direct descendants of the Pleistocene horses. They are of the same size and proportion as their ancestors, if we accept the evidence of the great numbers of fossil bones and skeletons of the latter which have been preserved at such sites as the Mendips in England, the Dordogne in France, and the terminal moraine of the Rhine glacier at Schussenreid in Germany. It is fortunate that they are not yet extinct, for although their blood is present in all modern horses, even in the Arabs, degeneration appears to be the ultimate fate of cross-bred horses, and only by use of pure original stock can regeneration be assured. This is not an original idea, and in fact was the subject of the report of a commission set up by the Secretary to the Ministry of Agriculture in 1912. In Britain we have the reduced remnants of original prototype

pony herds. In Germany, at Isernhagen near Hanover, it is being put to a practical test, and as a result of the deductions from 30 years' research, Exmoor ponies are to be used to help to form a regeneration herd for the production of agricultural horses. It may be pointed out that it is a requirement of this breeding enterprise that the horses must be able to live without any form of housing throughout their lives, and to require no subsidiary food if rough grazing is available, and the use of any form of hard feeding such as oats is not countenanced.

The modern Arabs are a very mixed lot as far as their dentition is concerned. Although under man's protection they are now so numerous, it is evident from the prototypes that they were localised in distribution and were not primarily grazing animals. Their cheek teeth, compared with the Northern horses, were relatively short-crowned, and the enamel pattern on the grinding surface was simple. Their incisors were very little curved, and the upper and lower incisors met each other at an acute angle. There was little depth in the mandible, and the maxilla—as might be expected from their dished faces—had no great height, so that even the short reserve crowns and roots could only get sufficient depth for their implantation by curving backwards. The result of this is that the molars at least, tended to have their long axes parallel with each other and if projected, would not meet at a point. The mechanical implication is that such horses had a less powerful masticatory action, a shorter effective life, and were less economical in feeding. The practical application is that the last molar wore obliquely and its grinding surface was therefore longer than that of the second last.

There is not much doubt, from the arrangements and proportions of the teeth, that these prototype Arabs were not developed for grazing on the coarse grass of the steppes and tundras, and indeed it seems likely that they were primarily browsing animals, and therefore probably of subtropical origin. They seem to have been small, perhaps eleven hands, and slenderly built. Crossed with the Northern horses they were greatly modified, and in time became numerous in domestication and extended their range as a consequence.

Because of the fact that many modern Arabs have been modified by crossing with Northern horses or ponies, it is extremely difficult to set absolute standards for their dentition, and in fact some of them have teeth more nearly of the Northern type. Those that have these Northern teeth cannot necessarily be considered fortunate, because the jaws that bear them have usually a tendency to be weak and slender like the prototype Arabs', and defects and irregularities of implantation and wear of the teeth consequently occur.

It is customary in Britain and America to talk of our " modern and improved breeds," when we really mean our artificial and specialised breeds. Whole races of hill and moorland ponies have been disposed of in Britain by the simple process of turning Arab or hackney stallions into the herds to replace the native stallions. The Manx, the Cushendal, the Tiree, the Long Mynd, the famous Galloways, the Goonhillys, and many others have been exterminated by this means, because

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the offspring of such crossings were so valuable that they were quickly sold out, but further crossings produced animals which were no longer able to fend for themselves as native Northern grazing horses.

Britain was once a land famous for its horses. We had the native stock then, just as we once had the native sheep and cattle. Imported foreign animals were grafted on to this stock, and from this temporary expedient, specialised breeds were produced which at least greatly improved on the imports, but lost much of the hardiness of the natives. Such a grafting technique is justifiable if we do not lose the native stocks with which to repeat the experiment when the pampered crossbreds begin to sicken. We can keep crossbreds alive, but it is an expensive process.

Teeth are only one of the factors to be considered in an estimate of how we have used the resources and wealth of native animal stocks which we inherited with our British Islands. Fortunately teeth and bones are durable structures and remain available for us to use in tracing history. By the use of X-rays we have managed to make something of an investigation into the progress and evolution of our horse breeds—or into their decline and fall.

The main purpose of this paper is to show that horses are of multiple origins. Since they are of multiple origins, it would be an error to believe that specialised breeds derived by cross-breeding can remain fixed and static in type. Even by careful selection within the breed, some factor will in time be missed, and degeneration will set in. In the past, famous breeds of horses were evolved in countries which had herds of native wild ponies. Examples of this can be seen in Syria, with its Kadeesh ponies of the Jebel, in France, with its Carmague ponies, in Poland with its Koniks, in Spain with the dun ponies of the Sierras, in Portugal with its garranos, and in Hungary. Last but by no means least, in Britain we were blessed with a great heritage of native ponies; in Yorkshire there were the Dales ponies, and consequently the famous Yorkshire coach horse and Cleveland bays, in Suffolk the Punch tills the soil in which his progenitor pony's fossil bones are numerous in the gravel pits, in Ireland the Connemara and the now extinct Cushendal ponies are in the ancestry of the famous hunters, and also in the "gyp's," and the thoroughbred owes not a little to the ponies of Exmoor, Dartmoor, Wales, and Galloway. These are merely a few instances.

The ponies came first. A long process of evolution produced them in nature's mould. Man quickly and easily converted them into horses by cross-breeding and selection, but then proceeded in spendthrift fashion to disperse or debilitate the raw material from which he could have reinvigorated these artificially-produced horses. Very little care would have saved the ponies. They are frugal and economical and need no domestication. It was only necessary to save them from exploitation and mongrelisation. The rest of the world believed that leisured and horse-minded Britain had saved its pure-bred ponies, and we have had the chance to do so. There is an imminent and sorry disillusionment in store for all of us on this point. A visit to the show ring can tell us why, for there it

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is patent that a true and valuable native pony has usually little chance against a showy, crossbred, but genetically worthless animal.

In Norway it is forbidden by law to cross-breed ponies, and no stallion can be registered or used for breeding unless it has successfully accomplished work and endurance tests.

Although Prjevalsky's wild horse does not resemble in skeletal detail either of the two Pleistocene ponies common to Britain and France, we have considered its mandible suitable for use as a standard for comparison in the sketches which follow.

Conclusions

There are two main sources from which our modern horses have been derived: these are, the Northern group developed on the grass plains immediately south of the Pleistocene Ice Caps, and the Southern group, which remained in a warmer, perhaps subtropical, environment.

The Northern group are specialised as grazing animals, and have long reserve crowns for their teeth, and deep jaws.

The Southern group more nearly resemble the horses of the subtropical Tertiary period, in having shorter reserve crowns, and are less suited for a purely grazing habit.

Horses are of multiple origins and degeneration is always a possibility in horses of mixed origins.

Some of the breeds of ponies still existing in Britain are of direct descent from the Pleistocene Northern group.

These prototype Northern ponies, although now so reduced in numbers, are vitally necessary for reinvigoration of our horse stocks.

Acknowledgments

Our grateful thanks are due to the Royal Scottish Museum for the use of the Alaskan material, and in particular to Miss Platt.

To the British Museum of Natural History for permission to photograph the Prjevalsky skull and the bones from the Mantell collection, and to Dr. Hopwood and Mr. R. H. Spires for the help they have given us.

To Mr. Balch of the Wells Museum for the Mendip material.

The Taunton, Scarborough, Reading and Brighton Museums, and the Department of Zoology of the University of Edinburgh have also generously made available the horse material in their collections, some of which has been used for the illustrations.

To Professor Boddie and Mr. Gregor of the Department of Medicine we are indebted for the X-rays, to Mr. R. S. Hood for the photography, and to Mr. E. Roberts of the Department of Anatomy for his technical assistance.

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Much of our information of the fossils of the Dordogne was obtained from Madame Lacorre.

Finally we would like to take this opportunity of expressing our appreciation to the German investigators whose enthusiasm and support have been invaluable to us. In particular we would mention Herr Ebhardt for his patient and practical pony-breeding researches at Isernhagen near Hanover, which have provided a practical background to our work, and Professor Dr. Heinz Tobein of the Geological-Palæontological Institute of the University of Freiburg who lent us the X-ray photograph of the Howenegg Hipparion.

OBSERVATIONS ON IMMUNITY TO CONTAGIOUS BOVINE PLEURO-PNEUMONIA, WITH SPECIAL REFERENCE TO THE BACTERICIDAL ACTION OF BLOOD

By F. W. PRIESTLEY

Sudan Veterinary Service, Khartoum

THE work recorded here commenced as a study of the bactericidal action of bovine blood for the contagious bovine pleuro-pneumonia organism; it was hoped that such a study would shed some light on the problems of immunity.*

Technique

Sterile blood, defibrinated with beads, was collected from the jugular vein and 1 c.c. amounts transferred to small, sterile, plugged tubes.

Loopful subcultures, before seeding, were made to 10 c.c. serum broths (as prepared by Bennett, 1932) as a check of the presence or absence of the pleuro-pneumonia organisms.

One loopful (4 mm. diameter) of culture was now seeded into the tubes of blood, which were incubated at 37° C. Loopful subcultures to 10 c.c. serum broths were made immediately and at intervals thereafter—usually daily for 5 days.

The day was noted on which growth occurred in the serum broths; all growth were confirmed microscopically, using stevenel blue (Priestley, 1952b). All negative serum broths were incubated for 10 days before being discarded.

To avoid contamination this technique was sometimes modified by the

* It will be clear that this work is incomplete. It is published now because the writer's contract with the Sudan Government is about to terminate, and in the hope that others more favourably placed will continue the work.

addition to the 1 c.c. blood, before seeding, of one loopful of penicillin solution containing 2,000 units per c.c. (see White, 1952).

The seed material consisted of a 48-hour culture in serum broth; older (7-day) growths were unsatisfactory in that growth was erratic and inconsistent even in control tubes subcultured immediately after seeding. There was little or no difference in the results whether virulent or non-virulent strains were used and, in consequence, non-virulent ones were preferred because they grow better.

Plasma from all samples of blood was tested for flocculation (Priestley, 1951a) and, from many, also for complement fixation (Campbell, 1938).

The Bactericidal Action of Blood

It became evident very early that there were marked differences in the bactericidal action of blood from immune, normal and dying animals. Specimen samples of results are shown in Table I. In general, blood from normal and dying animals (whether infected naturally or artificially) is without bactericidal action, whereas that from vaccinated or recovered animals is powerfully bactericidal, killing off the seeded organisms in less than 24 hours.

Table I.—Sample results of bactericidal tests.

Bull no.	Class	Unseeded control	Days seeded bloods incubated before subculture						Interpretation.
			0	1	2	3	4	5	
197	Immune	—	+3	—	—	—	—	—	positive
92	natural infection	—	+5	+5	+7	+7	+7	+8	negative
295	artificial infection	+8	+5	+5	+7	+5	+5	+5	negative
277	normal	—	+3	+3	+3	+3	+3	+3	negative

+3, +4, etc. = confirmed growth 3, 4, etc., days after subculture to serum broth.
— = no growth.

This bactericidal action varies considerably in intensity. Blood from recovered animals usually kills the seeded organisms in an hour or two, while that from vaccinated animals may take 24 hours or, sometimes, even longer.

The results of the 80 tests carried out are summarised in Table II.

It may be seen that of the 33 samples from normal animals only 8 showed any bactericidal action. It may be noted here that sera from some "normal" animals show faint traces of antibody insufficient to be recorded as positive to the flocculation or complement fixation tests; it is doubtless blood samples from these animals which show bactericidal action.

Of the 17 samples from vaccinated animals 12 showed bactericidal action. It is probable that the result of the bactericidal test in this group depends to some extent on how soon after vaccination the test is carried out; thus, of the 5 negative animals one was tested 5 and one 16 days after vaccination, and this is probably too soon for antibody production; there is, in fact, a suggestion

from the records that the test does not become positive until about 30 days after inoculation. It should be noted here that every sample positive to the flocculation test was also positive to the bactericidal test.

Table II.—The results of bactericidal tests on 80 blood samples.

Class	Flocculation reaction	Numbers tested	Bactericidal action	
			positive	negative
Normal	negative	33	8	25
Vaccinated	positive	10	10	0
	negative	7	2	5
art.inf. (recovered)	positive	11	11	0
art.inf. (died)	positive	15	1	14
nat.inf. (recovered)	positive	2		0
nat.inf. (died)	positive	2	0	2

art. inf. = artificial infection.
nat. inf. = natural infection.

Among the infected animals there was the most striking difference according to whether the animal survived or died. Of the 13 samples from animals which recovered every one showed a powerful bactericidal action. On the other hand, of the 17 samples from animals which died (or were killed moribund) only one showed bactericidal action; this animal was peculiar in that, subsequent to a test injection, it developed joint lesions from which the pleuro-pneumonia organism was isolated and only a transient swelling at the site of inoculation. It is important to note that serum from every one of these 30 animals gave positive flocculation and (where tested) complement fixation tests.

The Bactericidal Action of Plasma

With plasma the differences between the various types of animal are not so distinct. Undiluted plasma (or serum) from all cattle tends to be bactericidal to the pleuro-pneumonia organism. Nevertheless, marked differences do exist. Thus, plasma from recovered animals is generally bactericidal within an hour of seeding and such plasma can be diluted to at least 1 in 16 without losing its action; on the other hand, plasma from normal and dying animals takes 24 to 48 hours to exert its action and cannot be diluted at all without loss of bactericidal action.

The Mechanism of the Bactericidal Action

As might be expected, the bactericidal action of blood is independent of the cells and is dependent on the presence, in the plasma, of a specific heat-stable fraction (antibody) and a non-specific heat-labile fraction (complement).

If the washed cells from a normal animal are added to plasma from an immune animal the mixture is bactericidal; on the other hand, if the cells from an immune animal are added to plasma from a normal animal the mixture is not bactericidal.

Immune blood (or plasma) inactivated at 56° C. loses its bactericidal action, and this can be restored by the addition of unheated normal blood which, by itself, is not bactericidal (see Table III).

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The Similarity Between the Bactericidal and Flocculating Antibodies

Since sera from both recovered and dying cattle are positive to the flocculation test, and since blood samples from recovered cattle only show bactericidal action, it would appear at first sight that the two antibodies are different. In fact they are probably identical, as the following experiment indicated.

An inactivated immune plasma was absorbed with a heavy suspension of Campbell's flocculating antigen. After centrifuging, addition of unheated normal bovine plasma (complement) did not make a completely bactericidal mixture, presumably because antibody had been removed. That the lack of bactericidal action in the mixture was not due to fixation of the added complement (by circulating antigen-antibody complex not removed by centrifuging) was clear when it was shown that addition of the mixture to inactivated immune plasma caused the latter to become bactericidal.

Further experiments showed that the lack of bactericidal action in the blood of animals dying of pleuro-pneumonia was due to deficiency of complement. Thus, if to inactivated blood (or plasma) from a dying animal normal blood (or plasma) is added, the mixture generally becomes bactericidal. On the other hand, if to inactivated blood from an immune animal is added unheated blood from a dying animal, the mixture does not generally become bactericidal. Table III demonstrates these findings.

Table III.—The deficiency of complement in the blood of cattle dying of pleuro-pneumonia.

Mixtures of blood tested					Bactericidal action
immune		dying		normal	
unheated	heated	unheated	heated	unheated	
2 cc.					positive
	2 cc.				negative
		2 cc.			negative
				2 cc.	negative
	1 cc.			1 cc.	positive
			1 cc.	1 cc.	positive
	1 cc.	1 cc.			negative

This complement deficiency can be demonstrated directly. Bovine complement apparently has no lytic action on sheep red cells sensitised with rabbit H.I.B., but it does cause their agglutination. If to 1 c.c. volumes of sensitised red cells (0.5 c.c. of a 3 per cent suspension sensitised by the addition of 5 m.h.d. H.I.B. in 0.5 c.c.) are added 0.5 c.c. volumes of bovine complement either undiluted or diluted to not more than 1 in 4 and the mixtures are incubated at 37° C. in a water bath for one hour, agglutination of the cells occurs. This agglutinating complement can be demonstrated in the serum of normal and immunised cattle but not in that from cattle dying of pleuro-pneumonia infection. That the action is due to complement is suggested by the facts that heated normal bovine serum (56° C.) has no agglutinating action and unheated normal serum fails to agglutinate unsensitised red cells.

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The Mechanism of the Removal of Complement

(a) Removal by SSS or by whole organisms

The question now arose as to what causes the deficiency of complement in the blood of dying cattle. One obvious suggestion would be that some antigen-antibody complex circulating in the blood stream fixes the complement and removes it from the bactericidal system. The antigen in such a complex might well be something similar to soluble specific substances (SSS) or even whole organisms. In this connection it may be noted that nearly all animals dying of artificial infection with the pleuro-pneumonia organism show a bacteræmia at the time of and shortly before death; here, for instance, a bacteræmia has been demonstrated in 10 out of 14 dying cattle examined, and Nakumura *et al.* (1926), Hall and Beaton (1931), Campbell (1936) and others have reported similar findings.

That such a complex *could* remove complement sufficiently to upset the bactericidal action of a serum can easily be demonstrated. Thus, in one experiment, washed flocculating antigen was diluted in inactivated immune serum; 1 c.c. volumes were distributed to small tubes and 0.5 c.c. bovine complement added. The mixtures were incubated at 37° C. for half-hour before seeding and testing in the usual way. From the results shown in Table IV it will be seen that antigen diluted 1 in 2 and 1 in 4 completely abolished the bactericidal action of the mixture and that it was reduced even by a 1 in 8 dilution. It is to be assumed that this effect was due to fixation of complement since the amount of antibody absorbed by the antigen in the strongest dilution was quite insufficient to have any appreciable effect on the flocculation reaction.

Table IV.—Absorbtion of complement by an antigen-antibody complex.

Dilution of antigen	Days seeded mixtures incubated before subculture					
	0	1	2	3	4	5
1 in 2	+4	+3	+4	+3	+1	+1
1 in 4	·	+4	+6	+5	+6	·
1 in 8	·	+10	—	—	—	—
1 in 16	·	—	—	—	—	—
no antigen	·	—	—	—	—	—

· = not done.

This then *may* be the explanation of the complement deficiency in the blood of cattle dying of pleuro-pneumonia, but experiments on passive immunity raised another possible explanation.

(b) Passive immunity

There is no clear evidence as to whether cattle can be passively immunised against pleuro-pneumonia or not; experiments carried out here were inconclusive.

XUM

The Similarity Between the Bactericidal and Flocculating Antibodies

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			1 cc.	1 cc.	positive
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1 in 4	·	+4	+6	+5	+6	·
1 in 8	·	+10	—	—	—	—
1 in 16	·	—	—	—	—	—
no antigen	·	—	—	—	—	—

· = not done.

This then *may* be the explanation of the complement deficiency in the blood of cattle dying of pleuro-pneumonia, but experiments on passive immunity raised another possible explanation.

(b) Passive immunity

There is no clear evidence as to whether cattle can be passively immunised against pleuro-pneumonia or not; experiments carried out here were inconclusive.

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Four bulls were each injected with 50 c.c. serum from an animal which had recovered from an artificial infection ("immune" serum) and four more each with 50 c.c. serum from an animal dying of an artificial infection ("dying" serum). Simultaneously they, together with two controls, were given 1 c.c. 48-hour virulent culture on the opposite side of the body. Blood from the animal supplying the "immune" serum possessed a powerful bactericidal action; that from the dying animal had none. Five days later second injections of 50 c.c. appropriate serum were given and 5 days later still third injections. The serum injections were given subcutaneously and the virulent culture intramuscularly. The results are shown in Table V. One of the four animals receiving "immune" serum reacted very quickly and was killed moribund 20 days after the test injection; the other three showed nothing more than a transient reaction. All four animals receiving "dying" serum reacted and all were killed moribund. Both controls reacted; one was killed moribund and the other recovered. The difference in effect between the "immune" and "dying" sera is striking and, incidentally, meets the demands of statistical significance. However, there is no such difference between the animals receiving "immune" serum and the controls and it is not clear, therefore, whether the "immune" serum protects or whether the "dying" serum merely hastens death. It should perhaps be recorded that the sera used in this and other experiments had been stored over chloroform in the ice-chest for some time and were completely sterile; there was no question of virulent pleuro-pneumonia organisms having been injected with the "dying" sera.

Table V.—The results of one passive immunity test.

Bull no:	Serum inoculated	Reaction	Result
475	454 'immune'	—	S
503		swelling +++	K20
504		—	S
506		—	S
476	471 'dying'	joints ++	K20
478		joints +++	K34
502		joints +	K21
505		joints +	K21
507	controls	swelling +++	K30
508		swelling +	S

S = survived.
K = killed moribund.
Figures = days after inoculation.

Repetitions (somewhat modified) of the above experiment have produced very similar results. In all, 14 cattle have received "immune" serum and 8 have survived; 7 have received "dying" serum and all have died; there have been 8 controls and 2 have survived.

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From these results there is little doubt that there is a difference between "immune" and "dying" sera. But even the simple summation of the results between the immunised animals and the controls produces no positive answer in favour of passive immunity ($x^2 = 2.131$, which for $n = 1$ gives a value of "p" between 0.1 and 0.2, i.e., not significant).

Two further observations are recorded here without comment: (*a*) Animals simultaneously inoculated with virulent culture and "immune" serum on opposite sides of the body, if they reacted, developed swellings on that side receiving culture; those simultaneously inoculated with culture and "dying" serum developed swellings on that side of the body receiving the serum. (*b*) The swellings which developed in animals receiving "dying" serum were generally transient and were followed by a generalised pleuro-pneumonia arthritis from which the animal died; the pleuro-pneumonia organism can be isolated from these joint lesions in pure culture.

(*c*) *Removal by some other complex*

In spite of the above conclusion, the possibility of there being a second antibody had to be considered. Clearly, *if* there is any protection given by immune serum a second antibody different from the flocculating/complement-fixing antibody must be present since it would be palpably absurd to suggest that lack of protection with "dying" serum was due to deficiency of complement.

Table VI.—An attempt to demonstrate an anti-complementary mixture.

Dilutions of complement (1 cc.)	Mixed with	Days seeded mixtures incubated before subculture					
		0	1	2	3	4	5
undil.	heated 'immune' serum (0.5 cc.) and heated 'dying' serum (0.5 cc.)	+3	+1	—	—	—	—
1 in 2		+5	+8	+6	-	—	—
1 in 4		+7	.	+5	+9	+8	+9
1 in 8		+4	+6	+3	.	+7	+7
undil.	heated 'immune' serum (0.5 cc.) and heated normal serum (0.5 cc.)	+2	—	—	—	—	—
1 in 2		+5	+4	+5	+8	+7	—
1 in 4		+4	+4	.	+5	.	+10
1 in 8		+4	+4	+5	+5	+5	+9
undil.	heated 'dying' serum (0.5 cc.) and heated normal serum (0.5 cc.)	+10	—	—	—	—	—
1 in 2		+7	+2	+10	—	—	—
1 in 4		+3	+6	+4	+7	—	—
1 in 8		+5	+8	+5	+7	+6	—
undil.	heated normal serum (1 cc.)	+4	+5	+6	+8	.	—
1 in 2		+5	+6	+5	+5	.	—
1 in 4		+4	+4	+5	+5	+5	+6
1 in 8		+4	+4	+5	+5	+5	+7

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To test this possibility it was assumed that the antibody would be in excess in the serum of recovered or immunised animals and that the corresponding antigen would be in excess in the serum of dying animals. Mixtures of the two should then form a complex capable of fixing complement and removing it from the bactericidal system.

An illustration of the sort of experiment that has been tried is shown in Table VI. A series of 0.5 c.c. volumes of inactivated " immune " and " dying " sera were prepared; 1 c.c. volumes of various dilutions of complement were added and the mixtures incubated at 37° C. for one hour. The mixtures were then seeded with loopfuls of culture and tested for bactericidal action in the usual way. In this particular case there is the barest suggestion of some fixation of the complement. Thus, the immune serum and the dying serum alone (i.e., mixed with inactivated normal serum to give comparable bulks) when mixed with undiluted complement killed off the seeded organisms in under 24 hours whereas when the two sera were mixed this lethal effect took over 24 hours. Repetitions of this experiment using blood, serum or plasma have completely failed to demonstrate any complement fixation.

Discussion

There is nowadays ever-increasing evidence to show that immunity to certain chronic diseases, notably contagious abortion, tuberculosis and contagious bovine pleuro-pneumonia, is of an entirely different nature to that of immunity to acute diseases. Somatic (or surface) antigens and their corresponding antibodies do not appear to play any part in this immunity. It seems abundantly clear, particularly perhaps in the case of contagious abortion, that *living* organisms must be inoculated to set up an active immunity. Attempts to immunise cattle here against pleuro-pneumonia with organisms killed by heat (56° C., half-hour) or by chloroform or with organisms allowed to die out in culture have completely failed and these findings support those of Walker (1928, 1929) and Stewart (1935-36) who failed to immunise with formalin-killed organisms. The inference, therefore, is that immunity in such cases is dependent upon some *function* of the living organism rather than upon its structure. What that function is remains to be seen. As a working hypothesis it was considered by the writer that the organism, in producing its harmful effects, synthesises a " toxin " *in vivo,* that the body's response is a detoxification in the form of a protein linkage and that the protein concerned thus becomes virtually foreign and antigenic producing, in its turn, " antitoxin." It cannot be said that any evidence in favour of this hypothesis has been presented here. Nevertheless, the circumstantial evidence in favour of some functional activity of the organism is so great that it is hoped that this line of investigation will be extended.

Summary

The blood of animals recovered from contagious bovine pleuro-pneumonia is highly bactericidal for the causal organism.

The blood of animals dying of pleuro-pneumonia is not bactericidal and, in general, neither is blood from normal animals.

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Bactericidal action is dependent upon the presence of antibody and complement.

There is evidence to show that the lack of bactericidal action in the blood of dying animals is due to deficiency of complement and not to any deficiency of antibody.

Attempts to explain the deficiency of complement were inconclusive but suggest that there is circulating in the blood stream of dying animals some antigen-antibody complex which fixes complement and removes it from the bactericidal system.

* * *

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EQUINE MELIOIDOSIS IN MALAYA

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THIS paper records the occurrence of three fatal cases of melioidosis in race-horses between April, 1947, and June, 1949. Literature on the disease in horses is rare. Melioidosis was studied in Malaya by Stanton, Fletcher and Symonds,[1, 3] of the Institute for Medical Research, Kuala Lumpur, who demonstrated its occurrence in man, rats, cats, dogs, guinea-pigs and rabbits. They recorded that *Pfeifferella whitmori* was isolated from pus coughed up by an Australian race-horse in Malaya in 1925. The horse did not die. When destroyed eighteen months later no active lesions were found on post-mortem examination, nor was *Pf. whitmori* recovered from the organs. The deduction then made was that horses were highly resistant to the disease. The following histories may, therefore, be of interest.

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Case I

Subject.

Three-year-old English mare, imported into Malaya in November, 1946.

History.

The horse had been examined for insurance in late March, 1947, and had been passed as fit.

On April 19, 1947, it was withdrawn from a racing engagement because of a cough, and was first seen by one of us (J.D.) in Kuala Lumpur on April 25, 1947, showing a dry cough and an intermittent purulent discharge from the mouth. There was no nasal discharge and no palpable glandular enlargements. The temperature was 104° F. Appetite was normal.

Treatment.

The case simulated a strangles-like condition, at that time common among young imported race-horses, and the appropriate treatment was commenced, comprising an electuary and the administration of sulphanilamide. There was no response within three days, the temperature remaining elevated. Penicillin treatment was commenced on April 29, and was continued for four days, but it was without effect. Sulphanilamide was again resorted to for three days. On May 5 there was some improvement, and on the next day the temperature fell to normal and coughing ceased.

Careful examination of the head and chest gave no indication of the origin of the pus; the only abnormality was some slight pain on palpation of the pharynx and larynx. Appetite was normal throughout, although the animal was losing condition seriously.

On May 14 coughing recommenced and the temperature rose to 104° F. Febrifuges were administered without result, and the horse developed acute pneumonia and died on May 17.

Post-mortem Examination.

Abnormalities only are listed. Subcutaneous tissues: small hæmorrhages. Pharynx, nasal cavities, larynx and trachea: inflamed mucous membranes, but no ulceration. Lungs: acute pneumonia with many small abscesses throughout the lung tissue. One large abscess on the posterior border of the right lung had burst internally. Pleuræ: acutely inflamed. Heart: small hæmorrhages in the myocardium and endocardium. Lymph glands of the head, neck, thorax, axillæ and mesentery: acutely hæmorrhagic but no pus. Spleen, liver and kidneys: acutely hæmorrhagic.

Bacteriological Examination.

Samples of lung tissue were examined by the Institute for Medical Research, Kuala Lumpur, and *Pf. whitmori* was isolated.

Case II

Subject.

Five-year-old Australian gelding, imported into Malaya in late 1946.

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History.

The owner complained in Kuala Lumpur, on October 21, 1948, that the animal was suffering from incontinence of urine, and it was examined by one of us (C.W.W.). The temperature was 104.4° F. A continuous dribbling of urine from the sheath, which was reported to have been present for the previous three days, and a mild, infrequent cough, ascribed by the owner to a "chill" the horse had contracted a week previously during transit in heavy rain from an up-country racing engagement, were evident. Appetite was somewhat below normal, but had not caused concern. The conjunctivæ were a deep brick-red. A diagnosis of cystitis was made.

Treatment.

Penicillin was administered and half a grain of strychnine sulphate given to increase muscle tone in the bladder sphincter. A laxative diet was prescribed. Urine analysis gave a *pH* of 6.7, was negative for sugar and bile, but a trace of albumen was present.

An area of dullness low on the left side of the chest was detected that evening.

On the following day there was no significant improvement. The conjunctivæ were more injected and the incontinence of urine unchanged. Blood films were negative for protozoa. Hyoscyamus and pot. nitras were prescribed and one grain of strychnine was given, together with further injections of penicillin. The general picture was unchanged on October 23, the temperature being 104.4° F., and the conjunctivæ a muddy-red in colour, but urine incontinence was reduced. Penicillin treatment was continued, combined with sulphanilamide. On October 24 respirations were slightly abdominal in character; there was no incontinence of urine. The original diagnosis of cystitis had now to be abandoned, but no alternative one suggested itself. For the next two days the penicillin and sulphanilamide treatment was continued, but without any reduction of the temperature. By October 26 the horse had had 2,200,000 units of penicillin in oil and 12 oz. of sulphanilamide. On that day breathing became stertorous, and became worse as the day progressed. Chest auscultation by one of us (C.W.W.) and a colleague (Mr. W. E. Lancaster) revealed nothing other than an area of slight dullness low on the left side. On October 27 the horse was down in its stall. The eyes were sunken and the conjunctivæ wholly brown without any red tinge. Appetite was, for the first time, completely absent. The horse was reported to have given one cough about every three hours since the previous evening. Destruction was advised, and was carried out on October 28.

Post-mortem Examination.

Abnormalities only are listed. Urinary bladder: diffuse congestion of the mucous membrane of the fundus. Left lung: all surfaces covered with raised swellings from half an inch to six inches in diameter. Very little normal lung surface was visible. The largest swelling was incised and several ounces of yellowish-green pus gushed out. On irrigating the cavity it was found that all

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lung tissue from an area six inches in diameter on the parietal surface and to a depth of five inches had necrosed, leaving a cavity into which two fists could have been inserted with ease. It was assumed at the time that the case was one of bastard strangles.

Cultures from nodules were made at the Institute for Medical Research, Kuala Lumpur, and *Pf. whitmori* was isolated.

Case III

Subject.

Six-year-old Australian mare, imported into Malaya in 1946.

History.

The horse had been examined for insurance early in May, 1949, and was passed as fit, and had raced twice between May 21, 1949, and May 28, 1949, passing the official veterinary surgeon on each occasion.

It was reported as having a fever on June 14, 1949, and was seen by one of us (C.W.W.). The temperature was 105.8° F., and for the next six days was consistently elevated to between 104.8° F. and 106.2° F. The animal stood with all four legs tucked underneath it, but no signs of pain were evident. The anal sphincter was dilated and without tone and the rectum was ballooned. Blood films were negative for protozoa. Sub-acute colic was diagnosed.

Treatment.

Enemata and a colic drench were prescribed. There was little change on the following day, although there was some tenderness of the abdomen. On June 16, there being no improvement, penicillin was administered and was continued, in conjunction with sulphanilamide, on the following two days. On June 19 the conjunctivæ had undergone a marked change in colour from very slight injection to a dark reddish-brown. Moist râles were detected low down on the left side of the chest just above the level of the upper border of the heart. The anal sphincter had regained tone and the rectum was not ballooned. The animal was drinking copious quantities of water.

On reviewing the signal lack of success attending treatment, the suspicion arose that this might be another case of melioidosis. Remembering the lung lesions found at post-mortem in Case II, special attention was given to a chest examination, but no abnormality other than the râles already noted could be detected.

On the morning of June 20 the horse was recumbent, only semi-conscious, and was obviously about to die. The temperature was 106.2° F., pulse 70 and very feeble, and respirations 35 and stertorous. The conjunctivæ were much browner than on the previous day. It was decided to destroy the horse, but it died suddenly and quietly before this could be done.

Post-mortem Examination.

Abnormalities only are listed. The glandular portion of the gastric mucosa was deep purple in colour and necrotic, as also was the length of intestine from

the ileo-cæcal valve to the rectum. There were several pints of brown to clear fluid present in the lumen. Kidneys: enlarged to about one and one-third times their normal size and showed cloudy swelling. Lungs: grossly affected. The left lung showed numerous hard, discreet, raised lumps on all surfaces and scattered throughout its substance. These consolidated areas varied in size from an acorn to a closed fist and numbered about forty in the left lung. There were two large swellings, one corresponding in position with that area of the chest wall under which râles had been heard. The apex of the lung was pneumonic. Cheesy pus exuded from sectioned nodules and could also be expressed from the surrounding lung tissue and bronchioles. Each of the two large swellings contained 2-3 fluid ounces of pus in a stroma of necrotic tissue. The right lung was only slightly affected, containing some twenty nodules, all of a small size. The heart was slightly enlarged and the pericardial sac contained about 2 oz. of fluid. (There were no petechial hæmorrhages in any part of the carcass.)

Portions of the affected lung were examined at the Institute for Medical Research, Kuala Lumpur, and at the Veterinary Research Laboratory, Ipoh, and *Pf. whitmori* was isolated.

Discussion

These cases are three of the four which have been recorded in Malaya, the first case having occurred 22 years earlier. In the cases here described there were significant similarities—the refractoriness to penicillin and sulphanilamide therapy, multiple abscesses throughout one or both lungs, the absence or very limited presence of a nasal discharge, and the occurrence of a cough so mild and infrequent as not to attract attention. The points of difference between our cases are not significant and are attributable mainly to the degree of involvement. It is unfortunate that in none of the cases was the animal malleined, although the literature on the diagnostic value of mallein in equine melioidosis is scanty and conflicting. In none of our cases was a diagnosis made during life. Green and Mankikar[2] say, in connection with human cases: " Melioidosis is seldom diagnosed at the bedside; more often diagnosis is made in the laboratory or post-mortem room." The death rate for melioidosis in man is said to be about 95 per cent (Green and Mankikar).[2] Of the four equine cases reported in Malaya, i.e., the 1925 case and the writers' three, three have died.

It is not suggested that a positive reaction to a mallein test would have been of aid in treatment—the lesions were so gross that death was inevitable—but the opportunity to test the agent was missed, and is regretted. Chloromycetin (chloramphenicol) has been shown by Green and Manikar[2] to have an effect on *Pf. whitmori in vitro* not hitherto attained by any other drug or antibiotic, but under present conditions its very high cost would be a limiting factor in treatment, even if an early diagnosis could be made.

Our failures, until shortly before death, to detect the gross lung lesions by auscultation have caused us much speculation. It is just possible, we advance

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with some reserve, that the gross lesions developed in a fulminating manner within as short a period as two or three days. Discussions with medical colleagues, on the other hand, leave us in little doubt that without the assistance of X-rays the diagnosis of a lung abscess in the human subject can be attended with considerable difficulty. The interposition of several inches of normal tissue between lesions and stethoscope, as in our cases, might have been the reason for our failure to detect the abscesses.

We find it difficult to believe, from the pathology of the cases, that when the horses had raced four to six weeks before their deaths their lungs could have been involved to the extents that were seen at post-mortem. It is possible that infection had occurred months before (and even years, if the human analogy can be applied), and that their last racing engagement caused the breaking down of small foci, resulting in a generalised, fulminating spread.

The portal of infection in man or in animals is not known, and such evidence as there is is conflicting. Fletcher[3] records inability to infect horses with virulent cultures by intravenous, intranasal and subcutaneous inoculation, other than to cause temporary, localised suppuration, not leading to generalisation, by the latter route. Horses, he states, are unaffected by the ingestion of virulent cultures.

Rats are presumed to be the carriers of this disease. Although the premises where these horses, and many others, were stabled were not entirely free of rats, the numbers were, from all reports, exceptionally small.

Since infection by inhalation and via the skin cannot be ruled out, one of us (C.W.W.), having been exposed to gross infection in Case III, was advised to undergo a full course of chloromycetin treatment as a prophylactic measure.

Penicillin, unless used locally, if of little use in melioidosis; concentrations many times higher than that which could be obtained in the blood stream would have to be used.[2] Sulphadiazine or sulphathiazole appear to be the sulpha drugs of choice, the former being more effective *in vitro* and also being the less toxic of the two.[2]

Summary

Three fatal cases of melioidosis in race-horses in Malaya are described. They constitute, so far as is known, the only three equine cases recorded subsequent to the first case, also reported from Malaya, in 1925. The condition, in our three cases, was undiagnosed during life. Treatment was symptomatic.

Acknowledgments

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Senior Research Officer, Veterinary Research Laboratory, Ipoh, for his report on the reproduction of the disease in laboratory animals from material from Case III.

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OBSERVATIONS ON THE SURVIVAL ON PASTURE OF PREPARASITIC STAGES OF *DICTYOCAULUS VIVIPARUS* IN SOUTHERN ENGLAND—I.

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Introduction

THE control of parasitic diseases of the domestic animals is largely based upon two factors, namely, a sound understanding of the life history of the parasite, and the use of suitable anthelmintic. In the former respect, our knowledge is not always complete and there is not always a suitable anthelmintic available. However, the discovery of phenothiazine has been of much assistance in solving the problem of hæmonchosis by removing the adult parasites from the host. Unfortunately, no such effective remedy is available for dealing with lungworm infections of cattle. Thus, the control of the responsible parasites depends entirely upon controlled grazing in order to prevent re-exposure of the hosts. This, in turn, depends upon a sound knowledge of the bionomics of the free-living stages of these parasites.

As far as is known, *Dictyocaulus viviparus* can only maintain itself by propagation and infect succeeding generations of cattle in one of two ways. Either the parasite can survive in a free-living form on pastures over the long winter months, or be maintained during that time in the lungs of its host as the fifth stage form. In order to gain further information regarding the former possibilities, experiments have been carried out with regard to the factors involved in the survival of the larvæ on pastures. Such information as can be secured regarding this problem is essential in determining the length of time that cattle can be safely maintained on any particular piece of ground and subsequently return to it.

Two lines of investigation have been followed in this connection designed

mainly to find out more about the survival rate of *Dictyocaulus viviparus* on the pastures, viz :—

1. By making observations and carrying out certain tests on animals which were free of infection when allowed to graze on pastures naturally infected by cattle suffering from severe parasitic bronchitis.

2. By direct determination of the rate of survival of infective larvæ of *Dictyocaulus viviparus* on grass plots exposed out of doors.

In the former instances, observations were made upon *Hæmonchus contortus* and *Trichuris ovis* as these were already present on the infected field. The second experiment, however, was carried out with the free-living forms of *Dictyocaulus viviparus* collected artificially. These were applied under controlled conditions to specially grown plots of grass out of doors.

Historical Review

Three previous experiments on the survival of the larvæ of *Dictyocaulus viviparus* on pasture have been recorded. In 1941, Porter and his co-workers reported that yearling cattle failed to become infected with lungworms after grazing a pasture naturally contaminated five months previously with larvæ of cattle lungworm. In 1942, Porter published the results of two experiments. In the first, which was carried out during the summer, three calves each one month of age, were allowed a graze a naturally infected pasture for 43, 49 and 56 days respectively after the pasture was vacated. Fæcal examination and post-mortem findings of the three calves were negative. In the second experiment, carried out during the late spring and early summer, calves were grazed on experimentally-infected pasture. One contracted the infection seven days after the grazing started, but those allowed to graze 48 days after, failed to contract infection.

With regard to *Hæmonchus contortus,* Ransom (1906) reported that when larvæ were kept out of doors from December 27th to March 22nd, they showed little reduction in the number of food granules. In 1908, using sheep as experimental animals, he reported that pastures infested with larvæ retained their infectivity for 237 days at Washington. In 1910, he reported that if such pastures were vacated for a year they would become practically free from these larvæ. Monnig (1930) showed that the larvæ of this parasite lived 9½ months in sheltered pots of clay soil watered from the bottom and found that under natural conditions they lived for 3 months in soil in the dry weather of South Africa. Dikmans and Andrews (1933) reported that larvæ survived over the winter on pastures at Beltsville. Griffiths (1937) and Swales (1939 and 1940) showed that in southern Canada, pastures infested with *Hæmonchus contortus* were freed of infestations over the winter months although other Trichostrongylid larvæ were able to survive in small numbers. Shorb (1943), at Beltsville, Maryland, found that there was no survival on grass plots of *Hæmonchus contortus* larvæ from December to April. In 1944, he reported that few infective larvæ of this parasite were capable of surviving on infected grass plots from August to October to the following April or May and these were sluggish,

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vacuolated and probably non-infective. Sarles (1943) reported that *Hæmonchus contortus* larvæ failed to survive over the winter on pasture. On the other hand, Kates (1943) stated that larvæ of *Hæmonchus contortus* were capable of survival in small numbers on pastures over the winter. Dianburg (1944) at Beltsville, Maryland, experimenting with infective larvæ of this parasite also recorded that they lived for a short time on grass plots exposed out of doors and that they failed to live over the winter.

Baker (1939) reported that in the vicinity of Ithaca, New York, the infective stages of *Trichuris bovis* survived at least nine months. Hawkins, *et al.* (1944), working on the survival of sheep nematodes on pastures during the fall months, observed that those infested with *Hæmonchus contortus* became apparently free in two months in the late summer and early fall and that *Trichuris ovis* larvæ were still viable after $4\frac{1}{2}$ months on pasture. He concluded that permanent pastures in the generally accepted sense of the term cannot be blamed for perpetuating these parasites from one year to the next.

Material and Methods

A—Material

1. *The Experimental Site.* It was thought advantageous to carry out the experiment on a naturally infected pasture. Thus, a farm was selected where almost all the cattle had suffered from parasitic bronchitis in the summer of 1950. A total of 112 attested Ayrshire cattle (50 cows, 40 heifers, 20 yearlings and 2 bulls) were involved. They grazed intensively on two different parts of the farm. One section comprised 107 acres divided into 18, 9, 25 and 20 acres of two-year-old ley. A low-lying, damp place adjacent to these paddocks was fenced off. The second section some 3 miles away comprised 77 acres divided into four meadows (28, 20, 9 and 20 acres). This was an old park with the exception of the last 20 acres which was down to rape. The soil of the two sections of the farm was a light clay with a chalk sub-soil, 357 feet above sea level. The cattle were divided into three groups according to their age, namely cows, heifers and yearlings. While these remained separate units, they grazed over the same pasture from time to time. Husk was first observed among the yearlings towards the end of July, 1950. The heifers showed symptoms a week later and the cows towards the end of August. The 20 yearlings and 12 heifers that were the worst affected were brought to graze on the old park land on August 10 and kept under observation. Investigations were begun on August 12 after the first loss, when fæces examination showed the following:—

No. of samples	Cattle affected	No. of larvæ of lungworm per gm. of fæces	Parasite egg count	
			Trichuris eggs per gm. of fæces	Strongyle eggs per gm. of fæces
10	Yearlings	ranged between 28 and 500	ranged between 700 and 3,500	ranged between 100 and 500
5	Heifers	ranged between 20 and 100	ranged between 300 and 900	ranged between 100 and 200

Altogether four yearling died on this farm. Post-mortem examination was carried out on three of them on August 10 and 15 and September 8. They were heavily infested with lungworms and the three animals as a consequence had developed broncho-penumonia and died as a result. The parasitological findings on these yearlings are presented in Table I.

TABLE I

Parasitological Findings on the Three Dead Yearlings.

Date of death	No. of mature lungworms in lungs	Examination of digestive tract		
		Abomasum	Small intestine	Large intestine
10.8.51	662 *Dictyocaulus viviparus*	Heavy infestation with *Hæmonchus contortus*	Few *Hæmonchus contortus*	*Trichuris ovis*
15.8.51	873 „	„	„	„
8.9.51	451 „	„	„	„

It will be seen that in addition to *Dictyocaulus viviparus,* two nematodes were also present, namely, *Hæmonchus contortus* and *Trichuris ovis.*

2. *Experimental Animals.* As the farm on which the site was chosen was Attested, the experimental animals had also to be attested. Accordingly, four Shorthorn steers were chosen. They were all born during January, 1950, and were reared indoors; they had never shown any previous signs of coughing. Fæces examination on October 10, 15 and 20 failed to show any larvæ of husk worms in 200 grams of fæces by Baermann's technique, and were also negative for intestinal nematode eggs. As an additional check on the freedom of these animals from husk infection, they were submitted to an intradermal test with a polysaccharide antigen extracted from dried adult *Dictyocaulus viviparus*—negative results were, however, obtained. As work on this particular method of testing is in the experimental stages, this evidence can at the woment only be regarded as presumptive. The four yearlings were put on the chosen site to graze on October 25, 1950, that is, two months after the pasture was vacated.

2. *Method.* The four steers were left to graze until the experiment was terminated on February 1, 1951. Fæces samples of 200 grams were collected weekly and examined by Baermann's technique. Samples were collected for examination for evidence of lungworm larvæ eight, 12 and 16 hours later. An egg dilution count was also carried out on each of the four samples collected each week for evidence of intestinal nematodes.

Experimental Data

The results of the fæces samples collected from test steers are given below :—

Dictyocaulus viviparus. No evidence of the presence of larvæ was found

in any of the sixteen samples collected at weekly intervals commencing on October 25 and ending on February 1, 1951, in any of the four animals.

Hæmonchus contortus. No eggs were found in any of the above-mentioned samples.

Trichuris ovis. One hundred eggs per gram of fæces were first found in the four experimental steers on December 8, 1950. Subsequently, from 100 to 300 eggs per gram of fæces were found at the end of the experiment.

The following table shows the meteorological data from June, 1950, till January, 1951, and was collected from a site two miles from the farm. This period covers the time when husk infection occurred and the following experimental period.

TABLE II

Date 1950	Temperature Mean	Max.	Min.	Precipitation Total ins.	Snowfall ins.	Hours sunshine Month average	Daily average
June ...	62.0	72	52	1.79	—	245.0	8.2
July ...	61.5	69	54	4.22	—	187.1	6.0
Aug. ...	61.5	70	53	2.72	—	166.4	5.4
Sept. ...	56.0	63	49	2.87	—	105.5	3.5
Oct. ...	49.5	57	42	0.46	—	81.6	2.6
Nov. ...	49.5	57	42	0.46	—	81.6	2.6
Dec. ...	34.5	39	30	1.51	1¾	38.3	1.2
Jan., 1951	40.0	45	35	2.37	—	44.5	1.4

Discussion

Fæcal and post-mortem examination of the original members of the herd on the farm where the experiment was carried out established the fact that the pasture chosen was contaminated with the three parasites, *Dictyocaulus viviparus, Hæmonchus contortus* and *Trichuris ovis.* Weekly examination of fæces samples from the four experimental animals failed to reveal evidence of infection with lungworms or hæmonchosis. One steer died on January 4 from a *Cl. welchii* infection. Examination of its lungs failed to reveal any lungworm infestation.

Unfortunately, the digestive tract was not examined for evidence of hæmonchosis. The two intestinal parasites discovered on the site were, however, taken into account in the general experiment. The examination of fæces samples from the four animals, nevertheless, also remained negative for *Dictyocaulus viviparus* and *Hæmonchus contortus.*

Porter, in his first reported experiment of 1941, stated that closing a pasture for five months was long enough to sterilise it. In his second and third experiments the pasture was closed for 43 and 48 days, respectively, and again failed to maintain the infection.

In the present experiment the four steers were grazing on 10 acres and the grass was not lush. Whether the larvæ of *Dictyocaulus viviparus* and of *Hæmonchus cortortus* died and the field was sterilised, or whether they were still there just on the surface of the soil or round the roots of the grass, could

not be ascertained. Furthermore, it is interesting to speculate as to the possibility of the larvæ still being present, but being so old as to be incapable of causing infection. Nevertheless, under the conditions of the experiment the four steers failed to contract parasitic bronchitis or hæmonchosis.

As regards *Trichuris ovis,* the four animals showed evidence of infection after 40 days from the time of their exposure. This indicated that the two months under the conditions of the experiment were not enough to sterilise the pasture of *Trichuris ovis* larvæ.

Summary

1. Susceptible animals failed to contract lungworm infection when allowed to graze a naturally-infected pasture two months after being vacated by the infected cattle. The experiment covered the autumn and winter period from October 25, 1950, to February 1, 1951.

2. *Hæmonchus contortus,* which was recovered at post-mortem from the three animals of the original herd that were grazing the same site, also failed to become established in the experimental animals under the same conditions.

3. *Trichuris ovis* was able to reproduce itself as shown by examination of fæces from the four steers after 40 days grazing on the experimental pastures.

4. It would seem advantageous if a system of rotational grazing could be adopted where animals do not remain more than seven days on the strip grazed (i.e., the time known for first-stage larvæ to develop to the third stage). Furthermore, the same strip should not be grazed again before the lapse of at least two months.

DO SALMONELLA EXIST IN EGGS IN EGYPT?

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Introduction

Duck eggs have been the source of infection in outbreaks of food poisoning. Cases have been reported in many countries. To the best of my knowledge no outbreaks have been reported in Egypt.

I keep Sudanese ducks, and have done so for many years. The eggs are eaten by my family, and I have had no reason to suspect them as carriers of any toxic material.

Duck eggs are used in most of the large cake bake-houses in Egypt, and I have not heard of any intestinal trouble, following the eating of cakes, which could be attributed to Salmonella infection.

Literature

Topley and Wilson (1937) gave details of the classification of Salmonella antigenic and enzyme reactions, as well as the morphological and cultural characteristics and pathogenicity.

Bact. typhi-murium, a natural pathogen of rodents, particularly mice, is identical with *Bact. aertrycke,* which causes gastro-intestinal disorders in man. It is also identical with *B. pestis caviæ,* described by Wherry (1908), and causing epidemic enteritis in guinea-pigs. Finally, *Bact. typhi-murium* is indistinguishable from the organism isolated by Nocard (1893) from parrots that is a secondary invader in psittacosis virus disease.

Bact. typhi-murium is pathogenic for man as well as animals—mice and rats (Meyer and Matsmurak, 1927) and guinea-pigs—as well as parrots. It also causes epidemics in sheep (Bruns and Gasters, 1920; White, 1929; Lovell, 1932), chicks (Doyle, 1927), and pigeons (Beaudette, 1926).

It has been isolated from ducks' eggs (Scott, 1932-1933; Dalling and Warrack, 1932; Lovell, 1932), and was isolated from pigs that had died of swine fever (Lovell, 1932). A variety of *Bact. typhi-murium* was also isolated from a case of food poisoning in man and is not known to be a natural pathogen of animals (Schutz, 1920, and Kauffmann, 1931).

Bact. gallinarum is *not* motile and there are no flagella. It possesses only an O antigen which is identical with the O antigen of *Bact. typhosum* and *Bact. enteritidis,* and should be classified with them.

Bact. gallinarum varduisburg has been isolated from cases of gastro-enteritis in man (Muller, 1933), and is identical with *Bact. gallinarum.*

Bact. pullorum has been isolated from chicks, usually under three weeks of age, suffering from bacillary white diarrhœa (Rettger, 1900). Both *gallinarum* and *pullorum* are non-motile and are antigenically identical, but *pullorum* fails to ferment maltose, dulcitol or dextrin. *Gallinarum* does ferment these sugars.

Bact. pullorum is *not* known to infect man, and besides being pathogenic to chicks and fowls, it has been isolated from sparrows (Dalling, Mason and Gordon, 1925).

Bact. anatum has been isolated from cases of epidemic intestinal infection in ducklings (Rettger and Scoville, 1919-1920), and *Bact. anatum,* var. *muenster,* has been isolated from cases of food poisoning in man. The latter is antigenically identical with *Bact. anatum* only in one of its three groups, H. components. It also ferments inositol, while *Bact. anatum* does not.

Pathogenicity and Toxin Production.

Bact. typhosum.—Administration of typhoid bacilli by the mouth to ordinary laboratory animals (rabbits, guinea-pigs, rats and mice) does not give rise to any harmful result, but if large doses of living bacilli be administered intraperitoneally or intravenously a fatal infection is induced, and bacilli may be recovered from the blood.

Bact. typhi-murium.—This is a natural pathogen to laboratory animals. Mouse typhoid results after administration of small doses of living organisms by mouth or by inoculation. Septicæmia, usually associated with necrotic foci in internal organs, pneumonia and pleural exudate (Seifferet, Janncke and Arnold, 1928), are the pathological changes.

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Bact. enteritidis behaves more or less in the same way as *Bact. typhimurium*. White (1929) gives the following account of the group: a large genus serologically related, 1 to 3 microns by 0.5 microns, motile peritrichous, with few exceptions, failing to attack lactose and saccharose and failing to clot milk or produce indole, but regularly attacking glucose, with, and occasionally without, gas production. All known species are pathogenic for man or animals, or both.

Classification of the Salmonella types is a lengthy subject, but White sharply defined them serologically into two grades, namely:—

(1) Monophasic types, specific, typhosus, paratyphosus A, enteritidis Dublin, Derby, Moscow, *Abortus equi,* Dar-es-Salaam, and probably *Abortus ovis;* non-specific, European suipestifer, Boldgasen bacillus and the Binns strain of the *aertrycke* type.

(2) Diphasic types, paratyphosus B, Stanley, Bombay, *aertrycke,* Newport, Reading, *Mordificans bovis,* Sendai, Tokyo, Thompson, Hirschfeld, American suipestifer and Glasser's bacillus, also *sanguinarium*—such is a handy classification, but not decisive (White, 1926).

Viability.

Fletcher (1917) found that in a saline emulsion of fæces, paratyphoid A. B, survived over 100 days. Bumke (1925[1]) isolated paratyphoid bacilli from dried fæces after two years. *Bacteria suipestifer* has been isolated from dung after 36 days and from carcasses after 160 days, and from drinking water after 18 months. The gastric juice destroys typhoid and paratyphoid within a few minutes. The Salmonella group resist salt in pickled meat and are only destroyed on efficient pasteurisation of milk.

Serology: The Agglutination Test.

Bacillary suspensions in saline from moist agar growth, when there is less variation than in broth, are preferred. Chloroform was used as a preservative, as formalin should be avoided. For the study of somatic antigens, alcohol at 50° C. is added.

The Precipitation Test.

The readiest means is to extract the bacilli with hot dilute acetic acid (White, 1929).

Occurrence of Salmonella Bacilli.

Unlenhuth (1925) gives the view that Salmonella occur in nature, living and inert, in a saprophytic manner, and that from this saprophytic foothold morbid infection of man and animals by certain pathogenic races occurs from time to time. Bainbridge (1911) and Savage (1908, 1909, 1910, 1918) indicated that *Salmonella is essentially pathogenic,* but depends upon invaded hosts from which contamination originates.

Mice and rats, wild and tame, frequently harbour Salmonella, the guinea-

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pig is subject to latent infection, but the rabbit is less frequently so. Cattle, horses and sheep are not known to harbour Salmonella and there are no records of isolation of Salmonella from cats and dogs.

Flies are the main vehicles of carriage, but experimentally-infected flies might only harbour Salmonella for about one week.

Salmonella Infection of Birds.

Nocard (1893) recorded infectious enteritis in parrots under the name *B. psittacosis* (*B. aertrycke*). The strains isolated in America from ducklings under the title *B. anatum* (Rettger and Scoville, 1920) seem to be for the most part *B. aertrycke,* and there are *B. sanguinarium* and *B. pullorum.*

The main clinical features in affected birds are limp, drooping wings and disordered plumage. The birds appear dejected. There is severe diarrhœa, respiratory trouble, and rapid emaciation. In bacillary white diarrhœa, chicks usually die within 10 days of hatching. The main symptoms are diarrhœa and exhaustion. Post-mortem findings: Intestines pale and filled with slime, blotching of the liver with areas of focal necrosis, and petechial hæmorrhage. Mortality rate may be 90 per cent. Organisms may be recovered from the blood, intestines and viscera. Hens are ovarian carriers.

Preservation of Eggs.

Many methods have been advocated. The best method is to use the pickle prepared by mixing four parts by measure of fine slaked lime, 20 parts of cold water and one part of salt. A layer of cod-liver oil should be added to form a top film and avoid a crust. This may be ideal, following dipping of eggs for a few seconds in alcohol ether.

Experimental

(*a*) Ducks' Eggs.

A total of 60 eggs were examined. They were from various farms, namely, Faculty of Agriculture, Giza, Inshas breeding poultry farm, and eggs laid in my own garden. Forty-five ducks' eggs were laid by Sudanese ducks and the rest laid by various domesticated brown and white ducks. All these ducks were bred and reared in Egypt.

Experiment 1.

Five eggs, 20 days old, laid in clean nests, were incubated at 39° C. for three days, and then plated on McConkey media as follows:—

The incubator was fumigated before use. The shell was cleaned with cotton-wool soaked with absolute alcohol to which was added ether to make a ½ per cent dilution. With a sterile needle, 0.5 cm. in diameter, the shell was punctured and a sterile cotton swab introduced and well stirred inside, mixing the egg contents thoroughly. The swab was plated on the medium.

Plates were incubated at 37° C. for five days and examined. (This technique was followed in each experiment.)

Result. All five plates revealed no growth.

Experiment 2.

Five eggs, 35 days old, laid in clean nests, were incubated for three days at 39°C., then plated on McConkey media and incubated for five days at 37° C.

Result. Four plates were negative and one contained three pale, creamy, pigmented colonies of sarcina producing acid only in xylose, glucose, saccharose and maltose, but lactose, as well as rhamnose, arabinnose and inositol, were not fermented.

This sarcina 0.3 c.c. 48-hour-old broth culture was inoculated intraperitoneally into two white mice, with negative results.

Experiment 3.

Twenty eggs, newly laid, 2-5 days old. Ten eggs were soaked in absolute alcohol ether for one minute and then kept in a sterile container in an ice-chest for 40 days.

The remaining 10 eggs were kept uncovered on a shelf at room temperature (about 17° C.) and exposed to dust for 40 days.

They were all incubated for three days at 39° C. and then plated and incubated on McConkey media at 37° C. for five days.

Result.

(*a*) All the 10 cleaned eggs which were kept in the refrigerator were negative.

(*b*) Five eggs were negative.

(*c*) The remaining five eggs contained various sarcinæ, yellow, or creamy, or pink pigment, and yeast cells about 5 μ microns in diameter. These organisms separately proved non-pathogenic to white mice on intraperitoneal inoculation.

Experiment 4.

Thirty Sudanese eggs were used. They were kept at room temperature from 1-30 days as they were laid by two ducks, which I keep with an adult male. These eggs were incubated at 39° C. for 36 days, but not one hatched. They they were plated out on McConkey media and incubated for five days at 37° C.

Result.

(*a*) Ten eggs were sterile.

(*b*) Eighteen eggs revealed various sarcina and diplococci.

(c) Two eggs, besides sarcina, revealed non-lactose-fermenting colonies of bacilli. These colonies failed to ferment lactose, xylose, arabinose, inacatol, maltose, saccharose and mannite, and produced acid only in glucose. Subcultures produced acid in glucose, but failed to produce gas.

Morphology. Gram-negative, motile, slender, medium-sized organisms; non-capsulated, non-sporulating; morphologically indistinguishable from Salmonella.

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Experiment 5.

These non-lactose-fermenting, motile bacilli of five-day-old broth culture were inoculated intraperitoneally into:—

(*a*) Two white mice—0.3 c.c. each.

(*b*) Two white rats—0.5 c.c. each.

(*c*) Two ducks, one two months old and an adult—1 c.c. each, intravenously.

(*d*) Two rabbits—1 c.c. each, intravenously.

(*e*) Two guinea-pigs—1 c.c. each, intraperitoneally.

Result. All animals and birds were kept under observation for one month without any illness or mortality.

Experiment 6.

The same number of animals and birds were used and the same doses were administered, but the material was three-week-old culture filtrate on glucose broth *pH* 8 (Seitz filtrate). There were no pathogenic effects.

Experiment 7.

Three-day-old agar culture was emulsified in saline, and agglutination macroscopic test was carried out with the following immune sera, dilutions 1/25, 1/50, 1/100, 1/200, and 1/400. Controls used were:—

Immune sera against *Salmonella enteritidis H, Salm. newport, Salm. london,* and *Salm. poona 1, 6.*

Result. All dilutions used failed to agglutinate the organism isolated This organism looks like Salmonella, but is non-pathogenic.

Experiment 8.

Fæcal swabs from adult ducks, males and females, were plated on to McConkey media. Twenty birds were used, including the two ducks whose eggs contained the non-pathogenic organisms mentioned above. No Gram-negative, motile organisms; non-lactose fermenters were isolated; various cocci, sarcina; yeast and fungi were isolated. All proved non-pathogenic on intraperitoneal inoculation into white mice.

Conclusion.

1. The eggs examined which were laid by ducks in Egypt did not contain any Salmonella or any other pathogenic organisms.

2. Eggs laid in clean nests, and kept clean, proved sterile up to at least one month.

3. It is suggested that the shells of eggs which are for human consumption should be sterilised promptly with alcohol and kept covered. They should remain clean and fit for human consumption for at least one month.

(*b*) Hens' Eggs.

Sixty eggs were examined, 30 from the Faculty of Agriculture farm at Giza, 20 from Inshas, Doggi and Giza poultry farms, and 10 laid on my premises. Fowls were cross-bred Rhode Island and Astrolops with Fayoumi hens.

Experiment 9.

Ten eggs, which were laid on my premises. The nests were disinfected and sand and straw was replenished daily. These eggs, between two and five days

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old, were incubated at 39° C. for three days and then plated on McConkey media, as well as serum agar media.

Result. All 10 eggs proved sterile.

Experiment 10.

Thirty eggs were kept at room temperature for 20 days and then incubated and plated on two plates each of McConkey media and serum agar media.

These eggs included those from Doggi poultry farm, where *pullorum* disease was reported a year ago.

Result. No Salmonella or Salmonelloid (or *pullorum*) were isolated, but various sarcinæ and coli organisms, as well as yeast cells, were isolated, which all proved non-pathogenic to white mice on intraperitoneal inoculation.

Experiment 11.

Twenty five-day-old eggs were divided into two sections:—

(*a*) Ten were treated with alcohol ether and stored carefully in an ice-chest for one month and then incubated and plated as before mentioned.

(*b*) Ten eggs were kept exposed at room temperature for 30 days and then incubated and plated.

Result.

(*a*) Out of the 10 eggs treated with alcohol ether, eight proved sterile and two contained sarcina not unlike *Staphylococcus aureus.*

(*b*) All the 10 kept at room temperature contained various sarcina, diplococcus and coli.

Organisms isolated were non-pathogenic to white mice on intraperitoneal inoculation.

Conclusion.

Previous remarks regarding ducks' eggs apply also to hens' eggs. The problem of *pullorum* organisms in the egg of the infected or carrier hen will be dealt with fully in a separate paper, when only agglutination reactor eggs will be plated out. Previous experiments seem to indicate that *pullorum* organisms may gain access to the egg via shell pores.

(*c*) Geese Eggs.

Only 20 eggs were obtained, 10 from the Giza Faculty of Agriculture and 10 from various owners at Zeitoun.

The ten eggs from Giza were 10 days old, while the dates of laying of the others were unknown.

Geese-egg pores were minute and almost imperceptible, and the shell was very hard. The underlying membrane was thick and tough.

Experiment 12.

All 20 eggs were incubated and plated. All eggs proved sterile.

(*d*) Pigeons' Eggs.

Ten eggs were available at my own home and were left for three days under their hens. All were found sterile.

Summary

A number of eggs from Egyptian hens, ducks, geese, and pigeons have been

examined for the presence of Salmonella organisms. In no instance was a pathogenic organism recovered. (*Sal. pullorum-gallinarum* exist in Egypt and dealt with in separate paper.)

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REVIEWS

GENERAL SURGERY AND OPERATIVE TECHNIQUE (translated title), by A. Leuthold. Pp. 200. Basle: Ernst Reinhardt.

THIS book, of convenient size, commends itself mainly to the veterinary student, but could be read by veterinary surgeons with advantage. A second volume dealing with special surgery, by the same author, is to appear later.

The first part of the book covers the division of tissues and the healing and repair of surgical wounds, and includes the different types of sutures and knots used, with adequate illustrations. Local and general anæsthesia in all species of animal is dealt with in pages 49-76. The technique of producing joint and tendon sheath anæsthesia in the diagnosis of lameness is quite useful. The next 50 pages discuss the pathological aspects and treatment of accidental lesions and the more specific infections, such as botriomycosis and actinomycosis. The common neoplasms and cysts are briefly mentioned. Diseases of bones, joints,

muscles, tendons, tendon sheaths, bursæ, skin, blood vessels, nerves and lymphatic system are clearly and simply dealt with in sufficient detail for a book of this description, and the material forms an excellent clinical introduction to more specialised surgery. The final chapters comprise descriptions of the more common operations performed in the everyday life of the practising veterinary surgeon, male and female castration of all domestic species of animal, Cæsarian section, hysterectomy and neurectomy.

The author is to be congratulated on a very clear and lucid exposition of the elementary yet important aspects of clinical surgery. It is a book which can be well recommended.

POULTRY BREEDING. GENETICS AND SYSTEMS OF BREEDING. BULLETIN No. 146. MINISTRY OF AGRICULTURE AND FISHERIES. By G. E. Mann. London: His Majesty's Stationery Office. 1951. Price 3s.

THE science of animal genetics has made considerable progress within these last few decades. Breeders of poultry in this country, which has " the reputation of being the producer of the highest quality stock in the world," cannot afford to neglect its fundamental principles. A small, handy manual mainly intended for the breeders was a long-felt want in this field. The author of this book is to be congratulated for offering in the compass of 111 pages the broad outline of the various aspects of genetics which will meet poultry breeders' requirements.

The book is divided into five sections—the physical basis of heredity; poultry genetics; the inheritance of breed characteristics; the inheritance of economic characters; systems of breeding—and conclusion. As one would expect in a monograph like this, the details in some controversial topics in cytology, such as the number of chromosomes, are carefully avoided.

The reviewer is under the impression that the value of this book would have been greatly increased had there been a section dealing with the genetic aspect of the disease. A selected bibliography for further study would also be a welcome addition to it.

A useful glossary is appended. In spite of minor shortcomings, the book is, on the whole, highly instructive, and can be recommended to poultry breeders who would apply scientific principles to their craft.

THE USE OF TRACER ELEMENTS IN BIOLOGY, by W. G. Overend, Ph.D. Pp. 57. W. Heinemann, Ltd. (1951).

SOME sections of scientific and veterinary fields increase at such rapid rates that one needs to have lost contact with academic instruction for little more than a year or two before one's ideas are completely out of date. The use of tracer elements in biology is such a field, the recent advances in which are lucidly

described by Dr. Overend in a form which should not be found too technical by the average veterinarian.

Any author who attempts to describe, for the benefit of the non-specialist, recent advances in a specialised field, risks writing an account which is too difficult for the former class of reader and too simple for the latter. Dr. Overend does not always overcome this difficulty, his " Glossary," for example, defining some very simple terms, most of which should be familiar to his readers.

Nevertheless, in his early chapters dealing with the basic physical principles of tracers, in that describing the production and measurement of tracers, and in the final account of the use of tracers in biology and in medicine, the author has provided a short, simple account of his subject which veterinary surgeons generally should find time to read. The field is a fascinating one.

PHYSICAL VETERINARY MEDICINE (translated title), by Otto-Karl Eggart (1950). Pp. 185, illustrated. Stuttgart: Ferdinand Euke. Price 24 D.M.

THE book is to be published in two parts. The first volume deals with the physical and biological properties of hydro, thermal, light and radium therapy and their application to the multitudinous afflictions of the horse.

The first part of this volume covers the use of hot and cold water applications, inhalations, dry heat, massage, infra-red and ultra-violet light, radium therapy and the actual cautery. The various pieces of apparatus needed for these therapeutic measures are illustrated, and their mode of action explained. Many of them are used in human medicine, and it would appear that the " dosage " is judged by the degree of skin hyperæmia produced—a difficult means of assessment in most veterinary patients.

In the second part of the book hardly any defect, external or internal, is omitted which will not respond to the treatments outlined.

The book strikes a new note in the treatment of veterinary ailments, but one cannot help feeling that the author is a man of firm conviction, strong faith and of unbounded optimism. Faith and optimism have always been useful aids to the practitioner of physical medicine.

XUM

VETERINARY CONFERENCE

Ecole Veterinaire de Lyon announce the provisional programme for their conference to be held June 19-22, 1952, at Lyon:—

THURSDAY, JUNE 19

Morning: 9.00. Opening.

10.30. Professon Jean Blain: "The Pig—Rearing, Feeding, Zootechnics, Hygiene, Economic Importance."

Afternoon: 3.00. Dr. Joubert: "Recent Reports of Swine Fever in the Pig."

5.00. Professor Goret: "Recent Reports on Pig Pest."

FRIDAY, JUNE 20

Morning: 10.00. D. Bertrand: "Some New or Little-known Maladies of the Pig."

11.00. Meeting of the Association of Ex-students of the College of Lyon.

Afternoon: Reserved for Exhibition and Demonstrations in the Various Activities of the College and for Professional Meetings.

SATURDAY, JUNE 21

Morning: 9.30. Dr. Ferrando: "Microbiology of the Pig."

11.00. Professor Euzeby: "Parasites and Parasitic Maladies of the Pig."

Afternoon: Meeting: "Comparative Pathology," Under the Patronage of the Society for Comparative Pathology.

1. Professor Verge: "Animal Influenza, Pig Influenza."
2. Professor Lepine: "Human Influenza."
3. Professor Sokier: "Atypical Pneumonia Virus."

SUNDAY, JUNE 22

Morning: 10.00. Meeting of the Lyon Veterinary Science Society.

1. Dr. Fontenaille: "Inspection of Pork Meat—Tuberculosis of the Pig."
2. Professor Lucan: "Inspection, etc., of Pork Products."
3. Professor Coudbert: "Parasitic Maladies of the Pig Transmissible to Man."

Afternoon: 1.00. Banquet.

Afternoon Reserved for the Students of the College.

Evening: Annual Review by the Students, followed by a Ball.

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NOTICES

CALIFORNIA State Veterinary Medical Association. Annual June meeting. Arrowhead Springs Hotel, San Bernardino, Calif., June 16-19. Charles S. Travers, California State Veterinary Medical Association, 3004 16th St., rm. 208, San Francisco 3, Calif., executive secretary.

THE Distillers Company (Biochemicals) Ltd., Speke, Liverpool, 19, on behalf of its distributors: Allen & Hanburys Ltd., the British Drug Houses Ltd., Burroughs Wellcome & Co., Evans Medical Supplies Ltd., Imperial Chemical (Pharmaceuticals) Ltd., and Pharmaceutical Specialities (May & Baker) Ltd., announces the following reduced retail prices from April 28:—

Crystalline Penicillin G, Sodium salt and Potassium salt (buffered)—

	Single vial		Box of 5 vials	
	s.	d.	s.	d.
*0.1 mega unit	1	1½	5	7½
0.2 mega unit	1	4½	6	10½
0.5 mega unit	2	6	12	6
1.0 mega unit	4	1½	20	7½
Single container				
5.0 mega units	18	0		
10.0 mega units	34	3		

* Potassium salt (buffered) not available in this size vial.

'Distaquaine' G—	Single vial		Box of 5 vials	
0.3 mega unit	1	10½	9	4½
0.9 mega unit	4	7½	23	1½
			Box of 10 vials	
3.0 mega units	13	4½	133	9

'Distaquaine' Fortified—	Single vial		Box of 5 vials	
0.4 mega unit (3 + 1) ...	2	4½	11	10½
1.2 mega units (9 + 3) ...	5	10½	29	4½

'Distaquaine' Suspension—	Single vial		Box of 10 vials	
10 ml. (3 mega units) ...	14	0	140	0

Streptomycin (Sulphate) and *Dihydrostreptomycin* (Sulphate)—

	Single vial		Box of 5 vials	
1 gramme (1 m.u.)	4	3	21	3
5 grammes (5 m.u.)	19	0		

All prices subject to the usual discounts.

REGISTER OF BIOLOGICAL CONSULTANTS

THE Institute of Biology has recently established a Register of Biological Consultants. Subjects covered by biologists on the register include: Agronomy, bacteriology, biochemistry, ecology, entomology, fisheries, forensic biology, histology, microbiology, nutrition, pest control, protozoology, river pollution, and wild-life conservation. The Institute is now in a position to recommend specialist advisers in any branch of the biological sciences to those requiring their services. Enquiries should be addressed to the General Secretary, Institute of Biology, Tavistock House South, Tavistock Square, London, W.C.1.

Publishers' Notices

THE BRITISH VETERINARY JOURNAL, with which is incorporated THE VETERINARY JOURNAL, is published monthly, and copy for advertisements should be in the hands of the advertisement manager not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

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